retained cervical stumps is an established fact. I am satisfied, however, that it does not occur more frequently than in women who have not been subjected to operation.

I have tried to make plain the significance of the cervix as a factor in hysterectomy. In the selection of a surgical procedure where hysterectomy is indicated, the condition of the cervix is the answer. If its condition is beyond therapeutic cure, then its total removal, with that of other adjacent pathologic tissue, is necessary. I do not sanction its removal as a surgical routine. Where just cause exists its removal is imperative.

305 Medico-Dental Building.

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EARLY PREGNANCY—A HORMONE TEST FOR ITS DIAGNOSIS*

UTILIZATION OF RABBITS FOR TEST

A PRELIMINARY REPORT

By JOHN H. DORN, M. D.
JEAN R. MORSE, M. S.
AND
EDWARD I. SUGARMAN, B. S.
San Francisco

DISCUSSION *by D. Armstrong Taylor, M. D., San Francisco; R. Glenn Craig, M. D., San Francisco.*

INTRODUCTION

TO the rapidly accumulating corroboratory evidence on the Aschheim-Zondek [1] test as performed on rabbits after the method of Friedman,[3] we here submit a report on the results in 150 cases. After the publication of Friedman's [3] paper in the *American Journal of Physiology* in 1929, and also that of Schneider [4] in January 1931, suggesting the use of the rabbit in the Aschheim-Zondek test, we began our series, and having followed all but a very few cases through to a point where a definite diagnosis of pregnancy was possible, or where it could be ruled out, we are now prepared to submit our results. At this time many articles on the same subject are beginning to appear, but each paper is based on such a small series of cases that the total number of all reports falls far behind those which have appeared in support of the original Aschheim-Zondek test on immature white mice. Therefore, we present our series of 150 cases, with a few of our own practical suggestions, as further evidence of the accuracy and reliability of a test whose practical advantages are placing it ahead of the experiment as performed on white mice or rats. We hope that this evidence, plus that which we intend to offer in further experiments, will aid in the establishment of this test as the standard method for the hormone diagnosis of early pregnancy. These 150 cases are the first of our series; we have done almost 300 tests to date, but, as confirmations take time, we cannot at present report more. We hope to report on over 500 before the first of the year.

ADVANTAGES OF RABBITS

The advantages of using rabbits † are manifold, and there are practically no disadvantages. Large breeding stocks of white mice have to be maintained to furnish immature females of the proper age. It is an expensive, disagreeable, and precarious proposition. Mature female rabbits are purchased at any pet shop or poultry house at a reasonable figure. They are maintained in isolation for from fifteen to twenty days to make certain that the animal is nonpregnant, and to allow for the disappearance of old corpora hemorrhagica.[6] The problem of providing a suitable animal for the test is infinitely more simple in the case of the rabbit.

In the first part of our series we employed immature rabbits. However, since ovulation occurs only after copulation in the rabbit, the ovary of the mature nonpregnant rabbit which has been in isolation for a period of time sufficient to allow for the disappearance of the old corpora hemorrhagica, is just as satisfactory for observation as that of the virgin, immature rabbit.[2] Results are observed grossly, routine microscopic study being unnecessary, since corpora hemorrhagica are large and well defined. Results in our series were checked by microscopic sections for the purposes of experiment, but it is not at all necessary in routine clinical work. It is of immense aid to rule out the necessity of making microscopic sections on every case, from the point of view both of time and of expense.

A few minor difficulties were encountered. In the younger rabbits we occasionally made an error in determining the sex of the animal, since the external genitalia do not vary greatly in the two sexes. With a little experience, however, this

* From the Sugarman Laboratory.
* Read before the San Francisco County Medical Society, September 1, 1931.

† Editor's Note.—See article by E. Novak, Journal of the American Medical Association, June 27, 1931, p. 2175.

TABLE 1.—*Showing Results of Tests*

	Positive	Negative	Doubtful	Male	Dead	Summary
Number of results	67	60	8	11	4	150
Number clinically confirmed	13	54	....	....	....	114
Number of histories not obtained	4	3	....	....	....	7
Contradictions	....	3	....	....	....	3
Per cent error	....	....	....	....	....	2.5%

error is not often made. A few died in shock at the time of injection, although the urine was clear. We found that warming the urine just before injection reduced the mortality rate.

ARMAMENTARIUM

The test, in its simplicity and high degree of accuracy, should be of vast assistance, particularly to the small laboratory, and to the physicians in isolated localities. The apparatus required is almost primitive—a rabbit, a flask, a piece of filter paper and a funnel, a syringe and a needle. It is inexpensive, rapid (we allow the rabbit to remain alive for forty-eight hours), clear-cut in result, and dependable.

TECHNIQUE

Up to the present time 150 tests have been performed. In the first twenty-eight cases we employed the following technique:

A first morning specimen of urine was obtained and filtered. Seven cubic centimeters were injected into the marginal vein of the ear of a three months' old female rabbit. Twenty-four hours later the rabbit was killed and the ovaries examined for corpora hemorrhagica.

For the remaining 122 cases we made certain changes as follows:

First morning samples of urine were obtained and placed on ice for at least an hour to allow any precipitate to form. The urine was then filtered and placed in an incubator at 37.5 degrees centigrade to warm. Seven cubic centimeters were injected into the vein of a mature nonpregnant rabbit which had been in isolation for from fifteen to twenty days. Results were read in forty-eight hours after injection.

We consider the latter technique to be more satisfactory. In the first place, warming the urine reduces the mortality rate of the rabbits. Second, there is a danger of obtaining falsely negative results with immature rabbits if the rabbit is too young. The use of mature rabbits obviates this difficulty. Third, the 48-hour test allows more time for the development of the corpora hemorrhagica, and thereby reduces the number of false negative results.

In reading the results, we found three possible interpretations: (1) Positive, when there were present the large, fresh, bulging corpora hemorrhagica. (2) Negative, when the ovaries were small, flat, and showed no hemorrhagic spots. Enlarged Graafian follicles are not to be confused with the positive findings. (3) Doubtful, when small, flat hemorrhagic spots are seen. These are always to be repeated, like the uncertain results of any other scientific experiment.

In only two rabbits did we note the formation grossly of corpora lutea as well as corpora hemorrhagica, although microscopic sections showed lutein tissue in many of the latter.

Although this test is a macroscopic one (this being one of its most attractive features), we sectioned a sufficient number of ovaries to enable us to confirm the microscopic features. Sections of ovaries considered positive show corpora hemorrhagica with beginning luteinization. The longer the time after the injection the more luteinization appears.

Rheinhart and Scott [5] say that no luteinization appears before the forty-eighth hour, but we have found that it appears after twenty-four hours in slight degree. The interstitial glands may simulate corpora lutea, so this point must be borne in mind. In all cases the sections verified gross appearance, and in no case were sections necessary to determine the outcome.

The greater number of cases came through the courtesy of the obstetrical clinic at the University of California. The balance were sent to the laboratory by various physicians in San Francisco, chiefly from among the obstetricians. We have been able to obtain histories in nearly all of the cases up to the point where a definite clinical diagnosis was established.

RESULTS

The chart (Table 1) is self-explanatory in regard to the number of tests performed and the results obtained. The four animals which we lost died in shock at the time of injection. Of the eleven male rabbits used, three were used by mistake, and the remaining eight were tested with four known negative urines, and four known positive urines, respectively, to see if any change could be observed in the testes of the rabbits. There was no evidence of such change in this small series. The cases were not studied microscopically, however, and it is possible that small differences may have been present. The fact that such changes if they were present, were too slight to be observed grossly, immediately rules out the use of male rabbits, in the face of the immense advantage of the reading of the tests on female rabbits with the naked eye.

Included in our list of negatives were three male urines, three known negative female urines, and one on a rabbit which had received no injection. In all seven cases we obtained clear-cut negative results.

We received four postpartum cases. Three were taken twelve hours after delivery and all were positive in the rabbit. One was taken forty-six hours after delivery and gave a rabbit test which was interpreted as doubtful. In addition, with a urine from the case of an ectopic pregnancy obtained just before the operation performed to remove the fetus, we obtained a positive result.

An analysis of the results which we classed as doubtful show four cases which later gave positive results, two which proved to be clinically negative, and two on which we were unable to obtain any further information. We have, therefore, reached the conclusion that "doubtfuls" are to be repeated in every case, since our rechecks on these cases yielded a variety of results.

Our percentage error is entirely on the negative side and includes those cases which were tested in from one and one-half months' to four months' pregnancy; that is, cases where a positive result was to be expected. Following is a brief review of these three cases:

(1) Widmore			
2-11-31	4	months' pregnancy	Negative rabbit test
2-27-31	4½	months' pregnancy	Doubtful rabbit test
3-26-31	5½	months' pregnancy	Positive rabbit test
(2) C. B.			
1-28-31	4	weeks' amenorrhea	Negative rabbit test
3-9-31	2½	months' pregnancy	Positive rabbit test
(3) Case 135	1½	months' pregnancy	Negative rabbit test

Unfortunately the third case mentioned here was aborted, and we were not able to follow it to a point where a positive result was obtained, but it will be noted that in the other two instances positive results were eventually given. There may be some explanation for this fact in individual variation. Further experiments in the refinement of technique are necessary in order to reduce the percentage of error in the cases giving negative results so late in the term.

In the series are two cases which gave negative results in from two to three weeks after the menstrual period was due. They became positive very shortly after, as shown by this summary:

(1) E. D.			
1-22-31	2	weeks' amenorrhea	Negative rabbit test
1-28-31	3	weeks' amenorrhea	Positive rabbit test
(2) Case 146			
5-20-31	2	weeks' amenorrhea	Negative rabbit test

In a later series this test was repeated at three weeks past the period, and was negative. At six weeks it gave a positive result.

If these two cases are to be included in the list of contradictions, it would increase the percentage of error to 4.3 per cent. However, since most cases do not yield positive results any earlier than these, we feel justified in excluding them from our list of contradictions.

We have, on the other hand, obtained positive results at remarkably early periods of pregnancy:

1. Case 86 gave a positive result three days after a missed period.

2. Case 52 gave a positive result three days before the period was due, or between fourteen and twenty-one days after conception.

There is, as we have stated above, a certain amount of individual variation, depending, possibly, on the amount of hormone secreted in the urine, and in the earliest time that it begins to appear after conception in sufficient amounts to produce the reaction in the rabbit.

No falsely positive results were obtained. A very few gave the positive result at a relatively late period in the pregnancy. The highly specific results and the low percentage of error place the test in the first rank of laboratory tests.

CONCLUSIONS

1. The hormone test as performed on rabbits is accurate to a high degree.

2. The results are specific, giving no false positive reactions, and yielding only a very few negative results where a positive might have been expected.

3. The test is extremely simple to perform, and is thoroughly dependable as long as the proper attention is given to the selection and care of the test animal.

4. The use of the rabbit is highly desirable for the small laboratory or the physician in an isolated locality.

5. We urge the compiling of further data to aid in establishing this as the standard hormone test for pregnancy.*

450 Sutter Street.

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DISCUSSION

D. Armstrong Taylor, M. D. (490 Post Street, San Francisco).—To make a diagnosis of early pregnancy before the advent of laboratory tests was in many cases impossible, and even when there were some presumptive and probable signs present it was inadvisable to make a diagnosis of pregnancy for the following reasons: First, that the patient in some instances made false statements. Second, that cessation of the menses in a patient with a history of an irregular menstruation was not a reliable sign. Third,

* We wish to thank the Obstetrical Department of the University of California and the other members of the medical profession who so kindly coöperated with us in furnishing suitable material and in giving us the necessary case histories to corroborate our findings. We are also indebted to the University of California for the preparation of our microscopic sections.

that a woman near the menopause many times became pregnant without apparently giving any indication of pregnancy, believing that the cessation of menses was due to the menopause. Without positive signs, which are evident only from the fourth to the fifth month, a physician hesitated to make a diagnosis.

The advent of the Aschheim-Zondek test was one of the greatest contributions to the obstetrical art since prenatal care. It proved, in a small series of cases, that an early diagnosis of pregnancy could be made in not less than one hundred hours. Sterile technique, a microscopical examination, and the raising and care of a large number of mice or rats were the only requisites for accurate diagnosis of early pregnancy.

Eberson and Silverberg later reduced the time for the interpretation of the test to forty-eight hours by separating the ovarian hormones and concentrating the pituitary hormones.

These methods are, however, impractical as they require laboratory technique and a microscopical examination.

Friedman, in 1929, developed the rabbit test and with a small series proved that his method was equally as accurate as the Aschheim-Zondek method.

Doctor Dorn and his associates, with a much larger series, have proved conclusively that the Friedman test is accurate and simple to perform. No sterile technique is necessary and the test can be made in any office.

The great advantage of the rabbit test will be the early diagnosis of pregnancy in those patients whose welfare is at stake and in whom the continuation of pregnancy would be inadvisable. With further study, there is no doubt that the rabbit test will be the standard test for pregnancy in the future.

❦

R. GLENN CRAIG, M. D. (490 Post Street, San Francisco).—Since the first announcement of Aschheim and Zondek, reporting a high percentage of accuracy in the test for pregnancy which bears their name, numerous reports have appeared in the literature confirming their statistics. These have usually shown the test to be 95 per cent, or more, accurate, although Mazer and Hoffman report only 75 per cent accuracy. Attempts at modification of the original technique of Aschheim-Zondek have not given as good a result.

One objection to this test has been the four or five-day interval which must elapse before the results are known. Recently Eberson and Silverberg have proposed a quicker method, requiring thirty-six to forty-eight hours with equally good results.

Another objection to the use of immature mice or rats is the large breeding stock which must be kept on hand to insure a sufficient supply of immature animals. Since the rabbit has no regular recurring sexual cycle, as true ovulation only occurs after coitus (one of the few examples of economy in nature), the use of this animal would obviate this objection if the results were equally satisfactory. The results reported here speak for the accuracy, and are in agreement with other figures available when the rabbit has been used as the experimental animal. "Time will tell" which is most desirable.

Of course we must not forget that, to be of value, the report should include, or preferably be limited to, patients in whom the diagnosis of pregnancy is not easily made by digital examination. This would include both the early pregnancies, which should be less than two weeks after a missed menstrual period, and the abnormal pregnancies, such as an extra-uterine pregnancy, a pregnancy associated with myomati uteri, or the death of the fetus. Any effort to extend the accuracy of medical diagnosis, such as this, is to be commended.

CINCHOPHEN POISONING*

REPORT OF CASE

By EMIL BOGEN, M. D.
Olive View, Los Angeles

THE sad results of clinical disaster have furnished convincing evidence of the toxicity of many compounds which had been hailed as safe and harmless after the most exacting laboratory investigation. Cinchophen, a phenyl quinolin carboxylic acid, was produced in the course of researches in synthetic organic chemistry less than a half century ago.[1] Chemically related to the quinin derivatives, it possesses pharmacological similarities to the salicylates.[2] Its clinical value was suggested by the studies of its effect on uric acid excretion in 1908.[3] Since then its use has increased rapidly all over the world. It was early accepted by the Council on Pharmacy of the American Medical Association and incorporated in the Pharmacopeia of the United States.[4]

Elaborate pharmacological investigations by many workers, both in this country and abroad, consistently showed an absence of harmful effects from doses far exceeding any therapeutic expectations.[5] Most of the textbooks in use throughout the United States still assert that these preparations are practically devoid of danger.[6] Only in the last edition of New and Nonofficial Remedies is any mention made of possible fatalities from its use.[7] Clinical testimonials to its safety were also abundant.[8] Minor skin reactions were occasionally reported, but they were usually considered inconsequential rarities.[9]

Only during the past five years, it seems, have any deaths from this cause been recognized, but the continuously increasing reports of these fatalities leave little doubt as to their real existence and importance.[10] The actual number of persons who have used cinchophen derivatives is undoubtedly large, but when it is remembered that the usual indications for its use are often painful, but rarely fatal conditions in themselves, the incidence of fatal poisoning may not be disregarded. The following case report illustrates this fact.

REPORT OF CASE

A white woman, age nineteen, was admitted to the Olive View Sanatorium of Los Angeles County on December 17, 1930, and diagnosed as having "incipient tuberculosis." Her father and one sister had died of tuberculosis. She had always been a delicate child, had been severely burned at the age of one year, and again extensively burned over the entire right side and back when fifteen years of age. She had had measles, whooping-cough, chorea, chicken-pox and mumps, and her tonsils had been operated on twice under ether anesthesia. She had had typhoid fever at the age of six, and again four years later. Her appendix had been removed one year ago. She complained of anorexia, constipation, lassitude and undue fatigue, slight loss of weight, occasional pain in the chest, swelling of the left ankle, and slight elevation of temperature.

Physical examination revealed no signs of pulmonary pathology. There was a fluctuant swelling posterior to the left external malleolus, painful on walk-

* Editor's Note.—For brief statement concerning "Toxicity of Cinchophen and Safety of Neocinchophen," see Journal A. M. A., August 8, 1931, page 409. Also page 307, in this issue of California and Western Medicine.